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Some Properties of Intrinsic Factor-mediated Vitamin B_{12} Adsorption to Intestinal Mucosa Homogenate

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Summary

Distribution of intrinsic factor (IF)-mediated vitamin B_{12} adsorption capacity on the small intestine *in vitro* was found to be the same as that of vitamin B_{12} absorptive capacity *in vivo*. This strongly suggests that the attachment of IF-vitamin B_{12} complex to specific intestinal receptors represents a key step in the complicated process of vitamin B_{12} absorption. Moreover, it became evident that the presence of bivalent cations such as Ca^{++} and Mg^{++}, which are known to be necessary for vitamin B_{12} absorption *in vivo*, were essential at this step. The attachment of IF-vitamin B_{12} complex to the receptor seemed to require no energy.

Vitamin B_{12} is liberated from food by peptic digestion and bound by IF at acidic pH values in the stomach. The IF-vitamin B_{12} complex, being very resistant to digestion, is carried distally in probably almost unchanged form. The complex attaches to specific receptors (1–3) which are found in the brush border membranes (4) or microvilli (5) of the small intestine. The attachment of the complex to the receptor is in general thought to be the first step of intestinal absorption of vitamin B_{12} (–6–8). However, there are many unclear statements in the papers concerning the aspects of the attachment. One of the reasons for the unclearness is that many investigators utilize *in vivo* and *in vitro* systems in which it is difficult to distinguish between the attachment of IF-vitamin B_{12} complex to the receptor and the absorption of vitamin B_{12} through the intestinal mucosa. In our investigations described below, the intestinal mucosa homogenate system was used to omit the absorption through the mucosa. This method pointed out clearly the process of the attachment of IF-vitamin B_{12} complex to the intestinal receptor. In the first place, we confirmed the portion of the small intestine IF-mediated vitamin B_{12} adsorption occurs. In the second place, the effects of some factors such as temperature and bivalent cations on the adsorption of IF-vitamin B_{12} complex to the intestinal receptor were investigated.

Materials and Methods

The animals used in all experiments were Wistar strain male albino rats fed on commercial diet (NMF; product of Oriental Yeast Co. Ltd.), with body weight 250–400 g. In some experiments, animals were not fed overnight before the sacrifice. This is because of the state in which the food remains in the small intestine, occuping part of receptor sites by the complex of endogeneous IF and vitamin B_{12} with the food, and therefore the experimental value of attachment of IF-vitamin B_{12} complex may be decreased by a corresponding amount. In the experiments to investigate the distribution of IF-mediated vitamin B_{12} adsorptive capacity, rats were not fasted since the disappearance of food occurs at first in the proximal part of the small intestine and then proceeds distally. This causes the inconvenient phenomenon in which the fasting period determines on the location.

The rat intestinal mucosa homogenate was prepared almost as described by Castro and Glass (9, 10). Rats were sacrificed by decapitation. Immediately the small intestine was resected and freed from the mesentry with the aid of a razor. In the experiments to investigate the distribution of IF-mediated vitamin B_{12} adsorption capacity, the whole small intestine from the pyloric end of the stomach to the cecal valve was divided longitudinally into eight segments of equal length and the mucosa was scraped from each segment. In other experiments, the proximal half of the small intestine was discarded and only the distal half was used. The intestine was everted with a steel rod and the intraluminal content rinsed off in cold saline solution. The mucosa was scraped off with the aid of two microscopic glass slides. The scrapings were immersed in cold saline solution and homogenized in a Waring blendor at a high speed for 60 sec, then centrifuged at $1300 \times$g. for 10 min. The resulting sediment was washed once with saline solution and then resuspended in 10 ml of saline solution for each distal half of small intestine.

Rat gastric mucosa extract as the source of rat IF was prepared also as described by Castro and Glass (9, 10) with some modifications. Immediately after sacrifice of the rats by decapitation, the stomach was resected and opened along the small curvature. The intragastric content was washed out with running tap water and then with cold saline solution. The mucosa of the glandular portion was scraped with microscopic cover glass, weighed and homogenized in a Potter-Elvehjem homogenizer for 10 min, under ice-cooling. The homogenate was filled up so as 1 ml contained 20 mg of gastric mucosa, and centrifuged at $6000 \times$g. for 30 min at 0–4°C. The resulting sediment was discarded, the light opalescent supernatant as IF source was divided into small aliquots and stored in a freezer at –20°C until use.

The attachment of IF-vitamin B_{12} complex to intestinal mucosa was measured as follows (9, 10). To a 12×105 mm test tube containing 2 ml of Krebs-Henseleit bicarbonate glucose medium (11), the following were added in the order

listed: 1) 1 ml of appropriate dilution of IF source; 2) 0.3 ml of ^{57}Co-vitamin B_{12} solution containing 2500 μg of labeled vitamin B_{12} with specific radioactivity around 4 μCi/μg; and 3) 1 ml of a suspension of intestinal mucosa homogenate in saline solution which corresponded to 1/10 of the distal half of the small intestine of one rat. The tubes were capped by rubber corks and then incubated at 37°C for 1 hr with mechanical shaking. After incubation, the samples were centrifuged at 1300 $\times$g. for 10 min. The supernatant which contained an excess of unbound ^{57}Co-vitamin B_{12} were discarded by aspiration and the residues washed twice with saline solution containing $CaCl_2$ at 10 mM concentration. The washed sediments were counted in a well type scintillation detector. The vitamin B_{12} uptake by rat intestinal mucosa homogenate was recalculated in $\mu\mu$g vitamin B_{12} using ^{57}Co-vitamin B_{12} standards and in some cases, expressed as per cent enhancement of vitamin B_{12} uptake (E) produced by IF:

$$\text{E in per cent} = (U-C)\times 100/C$$

in which U is the uptake in the presence of IF, and C the control uptake with physiological saline solution in the absence of IF.

Results and Discussion

1. *Distribution of IF-mediated vitamin B_{12} adsorption capacity in small intestine*:

The whole small intestine from the duodenum to the ileum was divided into eight segments of equal length and numbered 1–8, beginning with the segment adjacent to the cecum as indicated in Fig. 1. The amount of IF-mediated vitamin B_{12} adsorption, which was obtained by subtracting the amount of IF-non mediated adsorption from total amount of adsorption, to intestinal mucosa homogenates prepared from each segment was measured. As shown in Fig. 1, there was a peak in segment No. 3, and No. 4 and No. 5 followed this. The portions with high IF-mediated vitamin B_{12} adsorption capacity such as segments No. 3, 4 and 5 corresponded to the mid-third of the entire jejunum and ileum which is known to be the main absorption site of vitamin B_{12} *in vivo* (12–14). The fact that the distribution of IF-mediated vitamin B_{12} adsorptive capacity *in vitro* agree precisely with that of vitamin B_{12} absorptive capacity *in vivo* emphasizes the adsorption of IF-vitamin B_{12} complex to the receptor site as the first step in the process of physiological vitamin B_{12} absorption.

2. *IF-mediated vitamin B_{12} adsorption to intestinal mucosa homogenate at different incubation temperatures*:

The IF-mediated vitamin B_{12} adsorption to intestinal mucosa homogenate were compareds when incubated at 37.0°C and at 1.5°C. Intestinal mucosa homogenate at 37.0°C adsorbed more vitamin B_{12} than at 1.5°C with various amount of IF added to the reaction system. However the adsorption at 1.5°C

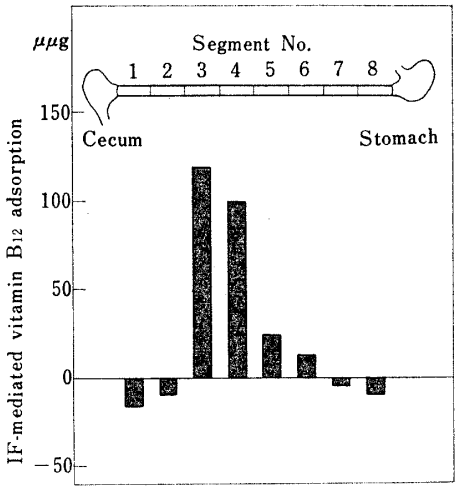

FIG. 1.

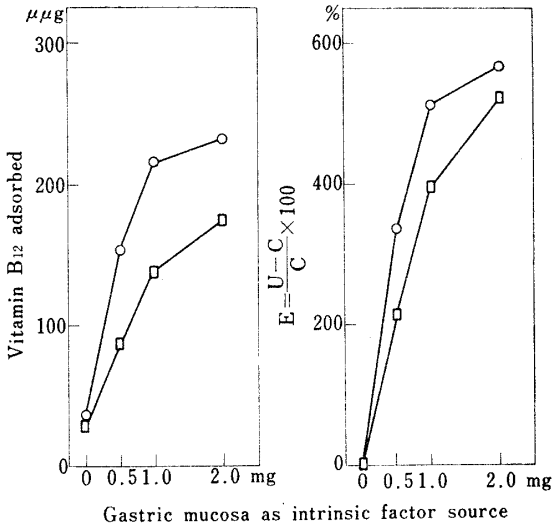

FIG. 2.

FIG. 1. Distribution of IF-mediated vitamin B_{12} adsorptive capacity in small intestine. Intestinal mucosa homogenates were prepared from each segments and IF-mediated vitamin B_{12} adsorption to each homogenate was measured. Incubation was performed at 37.0°C for 1 hr with mechanical shaking. The amounts of IF-mediated vitamin B_{12} adsorption were obtained by subtracting the amounts of IF-non mediated vitamin B_{12} adsorption from the total amounts of vitamin B_{12} adsorption.

FIG. 2. IF-mediated vitamin B_{12} adsorption at different incubation temperatures. The reaction condition was the same except for incubation temperatures, 37.0°C ○——○ and 1.5°C □——□ Other conditions were the same as in Fig. 1.

amounted to at least 60 per cent of the value at 37.0°C even when the difference was maximum. Moreover, in regard to the E value, which represents the functional activity of IF, it was not less than 70 per cent at 1.5°C compared with that at 37.0°C (Fig. 2). These results showed that the difference in incubation temperature exerted little influence on the IF-mediated vitamin B_{12} adsorption to intestinal mucosa homogenate. Hitherto there have been contradictory reports concerning the effect of a difference in incubation temperature on IF-mediated vitamin B_{12} adsorption to intestine. Herbert (8) and Donaldson *et al.* (5) reported that IF-mediated vitamin B_{12} uptake takes place even at low temperatures. On the other hand, in the report of Strauss *et al.* (15), it was shown that the IF-mediated vitamin B_{12} uptake and even adsorption to receptor site did not occur at 0°C at all but at 37°C. The experimental results in our study support the former. If the enzymatic or energy requiring processes are involved in the IF-mediated vitamin B_{12} adsorption, the reaction must not proceed at unphysiological temperatures such as 1.5°C.

3. *Effect of chelating reagent on vitamin B_{12} adsorption*:

The amount of vitamin B_{12} adsorbed to intestinal mucosa homogenate increased as the amount of IF increased in the absence of ethylenediamine tetraacetic acid (EDTA). But in the presence of EDTA at a concentration sufficiently high to

inactivate the total Ca^{++} and Mg^{++} ions which were contained in Krebs-Henseleit bicarbonate glucose medium, the addition of IF did not induce enhancement of vitamin B_{12} adsorption. The total adsorption had rather a tendency to decrease (Fig. 3). The reason for the decrease is thought to be that as the amount of IF increases, the formation of IF-vitamin B_{12} complex increases, and thus decreases the concentration of free vitamin B_{12}, which causes the reduction of IF-independent vitamin B_{12} adsorption, namely passive diffusion.

Hitherto, it has been known that EDTA inhibits the absorption of vitamin B_{12} *in vivo* (12) and that the inhibition is counteracted by the addition of Ca^{++}, Mg^{++} or Sr^{++} ions (16). It has also been recognized that IF-mediated vitamin B_{12} uptake by everted intestinal sacs *in vitro* is supressed by EDTA (17, 18). These *in vivo* and *in vitro* systems include two steps. The first step is the attachment of IF-vitamin B_{12} complex to receptor site and the following step is the absorption of vitamin B_{12} through intestinal mucosa. Thus it was obscure which step requires the bivalent cations. The results obtained in this study using intestinal mucosa homogenate confirmed the view (6) that bivalent cations are closely related to the adsorption of IF-vitamin B_{12} complex to the intestinal receptor.

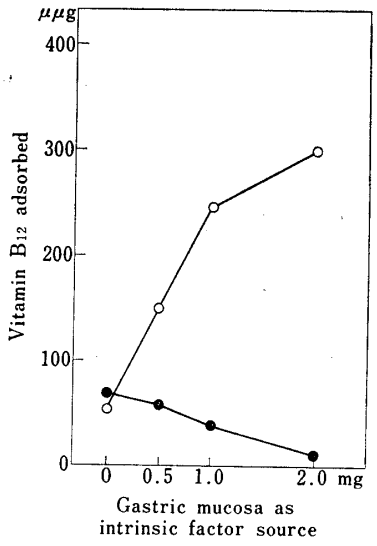

FIG. 3.

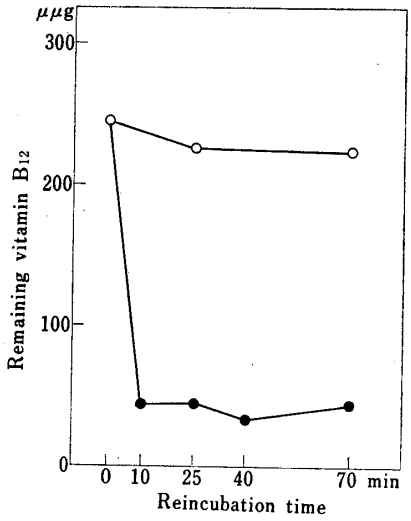

FIG. 4.

FIG. 3. Effect of EDTA on vitamin B_{12} adsorption. Vitamin B_{12} adsorbed in the absence of EDTA ○——○, in the presence of EDTA ●——●. The concentration of EDTA was sufficently high to inactivate the total Ca^{++} and Mg^{++} ions which were contained in Krebs-Henseleit bicarbonate glucose medium (7.4 μ moles/2 ml). Other conditions were the same as in Fig. 1.

FIG. 4. Effect of EDTA on release of vitamin B_{12} once adsorbed. Reincubated with EDTA ●——●, without EDTA ○——○. Reincubation medium added with EDTA contained sufficient EDTA to inactivate the total Ca^{++} and Mg^{++} ions which were contained in Krebs-Henseleit bicarbonate glucose medium. The time required for centrifugation to separate the intestinal mucosa from reincubation medium were included in reincubation times. The conditions of preincubation were same as in Fig. 1.

4. *Release of vitamin B_{12} from intestinal mucosa homogenate adsorbed previously with vitamin B_{12}:*

The intestinal mucosa homogenates which were once incubated with IF-vitamin B_{12} complex in the Krebs-Henseleit bicarbonate glucose medium were reincubated in the saline solution or in the saline solution containing EDTA at a concentration sufficiently high to inactivate the total Ca^{++} and Mg^{++} ions in the medium. In Fig. 4, the values of the remaining vitamin B_{12} at various reincubation times are shown. Although vitamin B_{12} is released to a little extent during the reincubation in the saline solution, the reincubation in the saline solution containing EDTA caused a release of a large amount of vitamin B_{12} within 10 min.

Herbert (8), using everted intestinal sacs, and Cooper *et al* (6), using intestinal perfusion method, reported that EDTA caused the detachment of vitamin B_{12} once uptaken. However, in these systems, not only IF-mediated vitamin B_{12} adsorption to the receptor site, but also absorption through the intestinal mucosa occurs during incubation with IF and vitamin B_{12}, and so the results obtained concerning the effect of EDTA were not so remarkable as in this experiment using intestinal mucosa homogenate.

We reported elsewhere (19) that the inhibition of adsorption of IF-vitamin B_{12} complex and the detachment of vitamin B_{12} once adsorbed by EDTA were not due to alteration of the function of the intestinal tissue by EDTA.

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